

elucidated that the 75kDa protein are located on the membrane of muscle fiber but the 150 kDa protein are localized at the Z-line of the sarcomere. The localization of these proteins are also confirmed by the transfection of GFP-fusion proteins into cultured skeletal muscle cells. These insights indicate that novel proteins that transcribed from the contiguous genes of connectin gene exist in striated muscles.

#### 2117-Pos Board B254

##### **Skeletal Myosin Binding Protein-C Isoforms Modulate Actomyosin Contractility and are Regulated by Phosphorylation**

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Myosin binding protein C (MyBP-C) is a thick filament-associated protein found in striated muscle and may regulate muscle contractility. Separate genes encode the fast and slow skeletal isoforms, and there are potential PKA phosphorylation sites in their functionally important N-terminal regions. Here, we compare mouse N-terminal fast (fC1C2) and slow (sC1C2) skeletal fragments containing the initial ~50 aa Pro/Ala-rich domain and the C1 and C2 Ig-domains that are linked by the ~100 aa M-domain. Of the known slow skeletal splice variants, we chose a highly expressed variant lacking the N-terminal 34-59 residue insert. To define the potential mechanisms by which skeletal MyBP-Cs affect contractility and whether these effects are modulated by PKA phosphorylation, we assessed the  $\text{Ca}^{2+}$ -dependent motility of rabbit skeletal native thin filaments over a surface of rabbit psoas myosin in the presence of C1C2 fragments. While thin filaments were fully regulated, with no motion observed at  $\text{pCa} > 7$  in the absence of fragments, the addition of either 0.50  $\mu\text{M}$  fC1C2 or sC1C2 resulted in significant motility. This suggests that skeletal MyBP-C isoforms effectively sensitize the thin filament. Under fully activating conditions ( $\text{pCa} \leq 5$ ), sC1C2 had little effect on motility whereas fC1C2 inhibited sliding velocities by nearly 50%. Thus, these fragments differ in their modulatory capacities with fC1C2 sensitizing the thin filament to  $\text{Ca}^{2+}$  and inhibiting maximal velocities, while the sC1C2 variant exhibits only a single mode of contractile modulation; i.e. thin filament sensitization. Interestingly, PKA phosphorylation in the Pro/Ala (sC1C2) and M-domains (sC1C2 and fC1C2), as confirmed by mass spectrometry, reduced both fragments'  $\text{Ca}^{2+}$  sensitization of the thin filament. Thus, the function of MyBP-C isoforms may be tuned to match the physiological demands of the muscle in which they are expressed.

#### 2118-Pos Board B255

##### **In vitro Reconstitution of Skeletal Muscle Contraction using Native Thin Filaments**

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Intracellular  $\text{Ca}^{2+}$  concentration regulate the muscle contraction by changing the conformation of Tropomyosin molecule over Actin filament. In this work we have purified native thin filaments (NTF) from Chicken Pectoralis muscle and reconstituted in *in vitro* motility assay (IVMA) with skeletal muscle myosin II. NTF prepared were  $1.67 \pm 0.14 \mu\text{m}$  in length and showed good regulation under different  $\text{Ca}^{2+}$  concentrations. At  $\text{pCa} 4$ , more than 90% of NTF were continuously sliding and when solution changed to  $\text{pCa} 9$  all the filaments were rigidly stuck to surface. Sliding velocity of NTF was  $V_F = 5.63 \pm 1.21 \mu\text{m/s}$  at  $30^\circ\text{C}$  and 17% higher compared to unregulated actin filaments. Fraction of sliding filament showed a direct correlation with  $\text{pCa}$  of buffer and  $\text{pCa}_{50} = 6.69$  was obtained using Hills equation fit. However the sliding filament velocity was not affected under different  $\text{pCa}$  concentration. Under varying substrate  $[\text{MgATP}]$  conditions in IVMA we found  $k_{\text{ATP}}$  is  $4.3 \pm 0.56 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  and  $k_{\text{ADP}}$  is  $404.33 \pm 2.7 \text{ s}^{-1}$  at  $30^\circ\text{C}$ . These kinetic parameters are comparable to values obtained using unregulated actin filaments, suggesting the detachment kinetics of myosin II is not affected due to  $\text{Ca}^{2+}$  regulation. We studied the effect of myosin density on thin filaments activation in IVMA. Even with fully activated thin filament ( $\text{pCa} 4.0$ ), decrease in myosin density leads to increase in fraction of stuck filaments. Decrease in fraction of motile filaments with myosin density in IVMA suggests there is minimum number of myosin head binding required to completely activate the NTF.

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#### 2119-Pos Board B256

##### **Direct Troponin-Myosin Interaction Enhances ATPase Activity of Cardiac HMM**

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Phenomenological evidence has long suggested that the strongly bound cross-bridges exert a positive feedback on myofilament regulation, which increases the sensitivity of thin filament to  $\text{Ca}^{2+}$  and achieves full activation. This positive feedback mechanism is uniquely important to the beat-to-beat regulation of cardiac output. Despite its importance, the molecular mechanism remains elusive. The current wisdom implies that the positive feedback mechanism is mediated by specific movement of tropomyosin on actin surface caused by actin-myosin interaction. Since the  $\text{Ca}^{2+}$ -regulated interactions between cardiac thin filament and myosin can be considered as an allosteric system which has long-range coupling between distinct components of the system, multiple protein-protein interactions at the interface between thin and thick filament may provide alternative mechanism for the feedback effects on myofilament activation. In this study, we investigated the potential interaction between troponin and myosin, known as troponin-bridge, and how the interaction affects function of myosin and thin filament regulation. The direct interaction between myosin and cardiac troponin was monitored using a sedimentation assay. Different reconstituted troponin complexes were incubated with myosin followed by ultra-centrifugation to pull down the myosin and the bound proteins. Western blot was used to identify the troponin subunits that were bound to myosin. Our results suggest that both N-cTnI(1-129) and C-cTnT (T2) bind to myosin, suggesting a possible direct interaction between myosin and IT arm of troponin. ATPase assay showed that the HMM ATPase activity was significantly enhanced by the presence of troponin regardless of the presence of actin and/or tropomyosin. In addition, truncation of the C-domain (residues 129-212) of cTnI also increased the HMM ATPase activity. The results of our study indicate that troponin-bridge may be a crucial component of thin filament regulation and play an important role in actomyosin interaction and muscle function.

#### 2120-Pos Board B257

##### **The Regulation of Actomyosin ATPase in Cardiac Muscle by the N-Terminal Extension of Cardiac Troponin T**

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Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) both have an amino-terminal variable region that plays a role in modifying the overall protein conformation and functions in the calcium-dependent regulation of cardiac muscle function. Previous studies have showed that in comparison with wild type controls, transgenic mouse hearts over-expressing cTnT lacking the N-terminal variable region (cTnT-ND) had an increased rate of relaxation. In contrast, transgenic mouse heart over-expressing cTnI lacking the N-terminal extension (cTnI-ND) had lower sensitivity to calcium activation of ATPase, resulting enhanced ventricular relaxation and Frank-Starling response. Recently, we demonstrated that the second order mant-dATP binding rate of cardiac myofibrils containing cTnI-ND was three-fold as fast as that of wild type myofibrils in low  $[\text{Ca}^{2+}]$ . The ADP dissociation rate of cTnI-ND myofibrils was positively dependent on calcium concentrations, while the wild type controls were not significantly affected. These results from native cardiac myofibrils under physiological conditions indicate that the N-terminal extension of cTnI plays a role in the calcium regulation of the kinetics of actomyosin ATPase. In the present study, we employed the same techniques in order to investigate which step(s) of the ATPase cycle is regulated by the N-terminal variable region of cTnT. mant-ATP binding, mant-ADP/ADP dissociation, and phosphate releasing rate of cTnT-ND myofibrils are examined and the results will provide novel information dissecting the function of the two troponin subunits and their posttranslational modifications.

#### 2121-Pos Board B258

##### **Pseudo-Acetylation of Actin Residues K326 and K328 Disrupts Drosophila Flight Performance and Muscle Structure**

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Striated muscle contraction is driven by cyclical interaction between myosin-containing thick and actin-containing thin filaments and is regulated by the